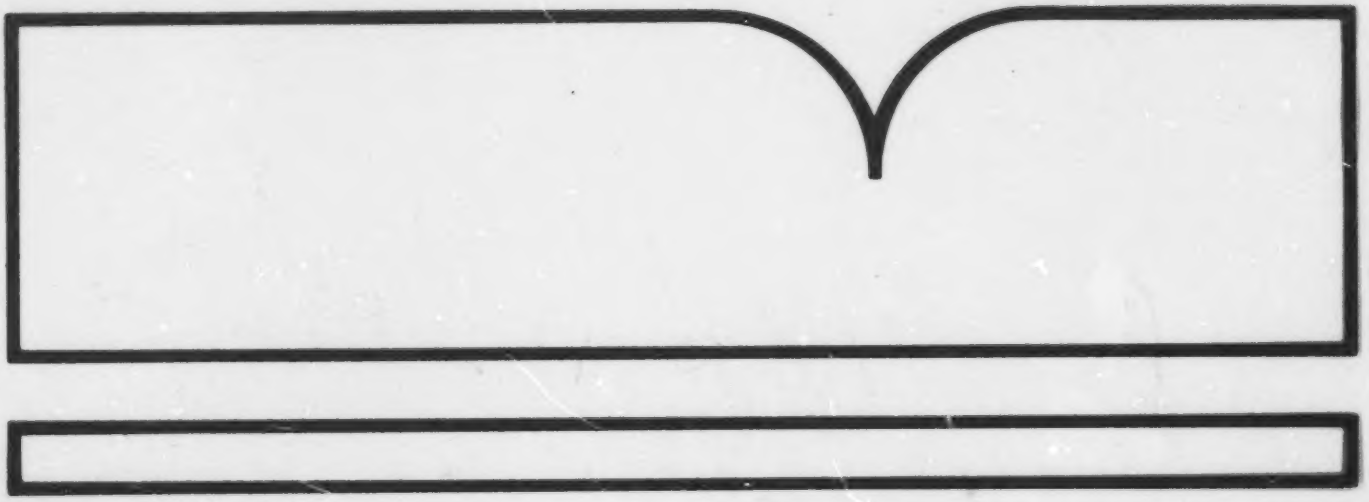
PB90-111626

Uptake, Distribution and Metabolism of Four Organic Chemicals by Soybean Plants and Barley Roots

(U.S.) Corvallis Environmental Research Lab., OR

1987



U.S. Department of Commerce
National Technical Information Service
NTIS

ERL-COR- 669J

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completir

1. REPORT NO. EPA/600/J-87/489	2.	3. R PB90-111626
4. TITLE AND SUBTITLE: The uptake, distribution and metabolism of four organic chemicals by soybean plants and barley roots		5. REPORT DATE 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): C. Mc Farlane,[1] C. Nolt,[2] C. Wickliff,[1] T. Pfleeger,[1] Ray Shimabuku,[1] and M. McDowell[3]		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: [1]USEPA, Corvallis, OR; [2]Cornell Univ., Ithaca, NY; [3]Oregon State Univ., Corvallis, OR		10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS: US Environmental Protection Agency, Environmental Research Laboratory, 200 SW 35th Street, Corvallis, OR 97333		13. TYPE OF REPORT AND PERIOD COVERED: Journal Article 14. SPONSORING AGENCY CODE: EPA/600/2

15. SUPPLEMENTARY NOTES: Journal of Environmental Toxicology and Chemistry, 6: 847-856, 1987

16. ABSTRACT

The uptake of bromacil, dichlorobenzonitrile (DCBN), nitrobenzene (NB) and dinitrobenzene (DNB) was studied in isolated barley roots and mature soybean plants. The uptake rate constants for soybean plants were in the order of DCBN = bromacil <NB <DNB. The same pattern was evident for the root tissue test, except for DNB. Metabolism of the ^{14}C-labeled chemicals indicated that DCBN was metabolized in the leaves but not in the roots. DNB was metabolized in the roots, and bromacil remained primarily as parent compound. A method for obtaining uptake rate constants is described.

Keywords: Plant uptake, Bromacil, Dichlorobenzonitrile (diclobenil), Nitrobenzene, Dinitrobenzene

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT: Release to Public	19. SECURITY CLASS (This Report): Unclassified	21. NO. OF PAGES: 12
	20. SECURITY CLASS (This page): Unclassified	22. PRICE: A03

EPA Form 2220-1 (9-73)

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL INFORMATION SERVICE
SPRINGFIELD, VA. 22161

EPA/600/J-87/489

Environmental Toxicology and Chemistry, Vol. 6, pp. 847-856, 1987
Printed in the USA. Pergamon Journals Ltd.

0730-7268/87 $3.00 + .00

Environmental Toxicology

THE UPTAKE, DISTRIBUTION AND METABOLISM OF FOUR ORGANIC CHEMICALS BY SOYBEAN PLANTS AND BARLEY ROOTS

CRAIG MCFARLANE,* CYNTHIA NOLT,† CARLOS WICKLIFF, TOM PFLEEGER, RAY SHIMABUKU and MIKE MCDOWELL‡

Toxic and Pesticide Branch, Environmental Research Laboratory, U.S. Environmental Protection Agency, Corvallis, Oregon 97333; †Environmental Toxicology Department, Cornell University, Ithaca, New York 14853; and ‡Soil Science Department, Oregon State University, Corvallis, Oregon 97331.

(*Received* 19 *August* 1986; *Accepted* 20 *April* 1987)

Abstract—The uptake of bromacil, dichlorobenzonitrile (DCBN), nitrobenzene (NB) and dinitrobenzene (DNB) was studied in isolated barley roots and mature soybean plants. The uptake rate constants for soybean plants were in the order of DCBN = bromacil < NB < DNB. The same pattern was evident for the root tissue test, except for DNB. Metabolism of the ^{14}C-labeled chemicals indicated that DCBN was metabolized in the leaves but not in the roots. DNB was metabolized in the roots, and bromacil remained primarily as parent compound. A method for obtaining uptake rate constants is described.

Keywords—Plant uptake Bromacil Dichlorobenzonitrile (diclobenil) Nitrobenzene Dinitrobenzene

INTRODUCTION

Many industrial and agricultural activities result in planned and accidental contamination of the environment with various xenobiotic chemicals. Since plants are a major component of every terrestrial ecosystem, it is essential that their role as vectors for fugitive anthropogenic chemicals to humans and other organisms be understood.

This study measured the uptake of four chemicals in excised barely roots and in mature soybean plants. Two representatives of each of two chemical types were used. Bromacil and dichlorobenzonitrile (DCBN) were selected because they are herbicides absorbed through roots [1,2] and translocated in plants. Nitrobenzene (NB) and 1,3-dinitrobenzene (DNB) were selected to provide data on the uptake and distribution of anthropogenic chemicals not used as herbicides. The two nitrogen-substituted benzenes are classified as "highly toxic" and of interest to the U.S. Environmental Protection Agency (EPA) under the Toxic Substances Control Act (TSCA, PL 94-469). DNB is an industrial chemical used in organic synthesis and dyes; NB is an industrial solvent that is listed as a priority pollutant by the EPA [3].

Anthropogenic solutes often enter plants through the roots, along with nutrients and water. The primary route is thought to include initial entry into the free space of the root tissue, followed by movement across a plasmalemma into the symplasm, then either into the phloem or xylem [4]. The membrane's permeability controls the solutes' entrance into the symplasm and thus is a selective barrier to solutes entering plants. Once inside, solute movement within the xylem is determined by both diffusion and mass flow [5,6], where diffusion is controlled by the energy potential of the solute and mass flow by the energy potential gradient within the continuous solvent (water) system. Movement within the phloem depends on diffusion, membrane transport and cytoplasmic streaming, and it requires metabolic energy.

Moody et al. [7] worked with exercised soybean roots in culture solution and found that the rate of accumulation of herbicides was greatest during the first hour, accounting for 50 to 70% of the chemical present in excised roots after 48 h. Of the total uptake during the first hour, 25% occurred in the first 2 to 5 min. McFarlane and Wickliff [8] found that the uptake of various xenobiotic chemicals by excised barley roots was linear following an initial rapid accumulation within the first 5 min of expo-

*To whom correspondence may be addressed.

sure. These data indicate that there is an initial, rapid uptake by excised roots, which is thought to be adsorption to the root surfaces, followed by uptake across the endodermis into the symplasm.

The uptake of organic substances by whole plants has also been studied extensively. It, too, has been observed to occur in two phases. First is a partitioning onto the external root surfaces and rapid accumulation of chemical into the free space of the roots [9,10]. Second, superimposed on the first, is a continuous but slower uptake into less accessible tissues of the root and passage across membranes [7,11,12], leading to transport to the shoots [13]. Once within the conductive tissues, chemical movement in the xylem is influenced by water movement, which is controlled by the water potential gradient created in the leaves. This is a major factor that is absent in the tissue test.

Many chemicals are thought to be taken up passively in plants. In such cases, movement of chemicals from soil solution to the leaves can be seen as partitioning between soil water and root tissues, and if the chemical can pass into the endodermis, the partitioning continues between the transpirational stream and the tissues of the stems and leaves.

Our goals were to obtain some basic information on how plants may interact with very low levels of NB and DNB in solution; to compare the uptake and translocation of these two anthropogenic chemicals with that of bromacil and DCBN; and to compare uptake rate constants from two different experimental systems (whole soybean plants and excised barley roots) to determine if such comparisons are understandable and useful.

MATERIALS AND METHODS

Test chemicals

The four, ^{14}C-ring-labeled test chemicals were bromacil (5-bromo-3-*sec*-butyl-6-methyluracil), diclobenil (2,6-dichlorobenzonitrile, DCBN), nitrobenzene (NB) and 1,3-dinitrobenzene (DNB). These commercially available chemicals (Pathfinder Laboratories Inc., St. Louis, MO) were dissolved in a small amount of either ethanol or benzene upon delivery. The specific activity and purity (which exceeded 98% in every case) were checked by GLC, TLC and liquid scintillation counting (LSC) techniques. All reported ^{14}C activities (dpm) were measured in a Packard Tricarb 460C liquid scintillation analyzer. Dosing solutions were made up with water and added to the hydroponic solutions in the exposure chambers through a sampling and injection port to give the specific activities and initial concentrations presented in Table 1. Low concentrations were chosen to prevent visible damage to the plants and to give levels of radioactivity suitable for the detection techniques used.

Isolated root uptake test

Chemical uptake was tested in isolated barley (*Hordeum vulgare* L.) roots using a previously described method [8]. Briefly, roots from 5-d-old hydroponically grown barley plants were cut, and small aliquots were placed for 3 to 27 min in nutrient solution containing the test chemical. Roots were then rinsed for 30 s in fresh, isotope-free nutrient solution, blotted between two sponges, placed in a paper container and oxidized. The $^{14}CO_2$ generated by oxidation was collected in liquid scintillation cocktail and the ^{14}C activity was determined by LSC [14].

The rate constants of chemical uptake were derived from a linear regression of ^{14}C activity in roots (expressed as dpm/g dry weight) divided by solution ^{14}C activity (dpm/ml) against time. The rate constant (slope) represents the fractional uptake per minute of chemical from solution to roots [8].

Whole plants

Chemical uptake was determined in mature, hydroponically grown soybeans (*Glycine max* [*L.*] dwarf cultivar, Fiskeby V). The plants were grown in a hydroponic nursery in a greenhouse for 40 d and then moved to the controlled environment in the exposure chambers, where they were held for 3 d before experimentation. Contamination experiments were conducted in specially built exposure chambers designed to contain toxic chemicals while measuring environmental conditions and some plant functions [15]. The atmospheric conditions of the tests were as follows: photosynthetic photon flux = 350 μmol s^{-1} m^{-2} (continuous); temperature = 25°C ± 1°C; atmospheric moisture = 12 g m^{-3}; carbon dioxide = 15.6 mmol m^{-3}; air velocity at the top of the plant canopy = 0.6 m s^{-1}. The nutrient solution was a modified, half-strength Hoagland's solution [16] with pH 6.0 and an electrical conductivity of 1.2 dS m^{-1}. Under those conditions, the average CO_2 assimilation was 0.19 mmol plant^{-1} h^{-1} and the average transpiration rate was 5.6 ml plant^{-1} h^{-1}. Eight plants were placed in each exposure chamber. For the bromacil and NB experiments, three

Table 1. Initial solution concentrations and specific activities of test chemicals in the hydroponic solutions in the exposure chambers

Chemical	Concn. (μg/L)	Specific activity (mCi/nmol)
Bromacil	86	16.3
DCBN	45	16.9
NB	69	26.1
DNB	34	30.2

DCBN, dichlorobenzonitrile; NB, nitrobenzene; DNB, dinitrobenzene.

chambers containing plants were run concurrently. The data presented here are from only one of these chambers per experiment because the other chambers were controlled at different transpiration rates. For DNB and DCBN, the data from two chambers containing plants are presented. Only one test chemical at a time was run because of limited laboratory space, complex procedures and extensive plant sampling procedure. Unfortunately, replicates of the NB and bromacil experiments were not performed, but the data obtained are still important.

Hydroponic solution sampling

At various times following dosing, 1-ml samples of the hydroponic solution were removed from each chamber, added directly to scintillation cocktail (either Aquasol or Optifluor) and counted for ^{14}C activity. In earlier experiments, three replicate samples were taken at each sampling time, but because there was so little variation (coefficient of variation of less than 0.5%), single sampling was adopted. Also, the sampling schedules were different for various chemical tests to reduce the work needed to retrieve the required information.

Data presentation

Chemical uptake into plants was evaluated by two methods. The first involved measuring the loss of chemical from the treatment solution; the second, measuring the uptake of chemical into the plant. All materials in contact with solution were selected to minimize adsorption so that the loss of chemical from solution would reflect the plant uptake rate. Most of the contact surface area was of ultra-high-molecular-weight, high-density linear polyethylene. All parts of the pump in contact with liquid were constructed of Teflon or stainless steel. Nevertheless, an exposure chamber without plants was included in each experiment to allow determination of adsorption of chemical to chamber walls.

The rate of uptake, as calculated by the first method, was estimated using a theoretical model. The model is based on mass balance theory as follows. The rate of change of chemical in the tank solution at an elapsed time (t) was described as a combination of two factors: the rate of uptake by the plants and the rate of sorption to tank walls. Each of those factors has two components: for plants, movement into and out of roots; for tank walls, adsorption and desorption. By using the initial portion of the uptake curve, back transport from the plant into solution is assumed to be negligible, leading to the following equation (see Table 2 for symbol definitions):

$$V_t \frac{dC_t}{dt} + V_s \frac{dX_s}{dt} = -\lambda_f C_t(t) \quad (1)$$

rate of change of chemical in the tank	rate of change of sorbed chemical in the tank	uptake rate into the plant

Table 2. Definitions for rate constant equations 1 through 8[a]

Symbol	Definition
V_t	Volume of the main tank, the plant containers and fittings (ml)
V_s	Volume of the monomolecular layer for sorption of chemical onto tank walls (ml)
C_t	Chemical concentration in the tank (dpm/ml)
λ_f	Forward rate of chemical uptake by the plant, unique for each chemical (ml/min)
K_r	Desorption rate constant (1/min)
K_f	Adsorption rate constant (1/min)
γ	K_f/K_r = Pseudo-partition coefficient (dimensionless)
X_s	Concentration of sorbed chemical in the tank (dpm/ml)
C_0	Initial concentration of chemical in the tank (dpm/ml)
M_r	Total chemical in plant tissue

[a]See the text for equations.

where

$$\frac{dX_s}{dt} = K_r(\gamma C_t - X_s) \quad (2)$$

is the rule of first-order kinetics of sorption.

Equation 2 was integrated from time 0 to t to obtain:

$$X_s(t) = K_r\gamma \int_0^t C_t(\tau)e^{-K_r(t-\tau)}d\tau \quad (3)$$

Assuming that $X_s(0)$ and $C_t(0)$ equal zero, and applying a Laplace transformation, Equations 1 and 3 can be solved to obtain an expression for $C_t(t)$:

$$C_t(t) = C_0\left(\frac{K_r + S_1}{S_1 - S_2}\right)e^{S_1 t} - C_0\left(\frac{K_r + S_2}{S_1 - S_2}\right)e^{S_2 t} \quad (4)$$

where

$$S_1 = \frac{-\left(K_r(1+\phi\gamma) + \frac{\lambda_f}{V_t}\right)}{2} + \frac{\sqrt{\left(K_r(1+\phi\gamma) + \frac{\lambda_f}{V_t}\right)^2 - 4\frac{\lambda_f K_r}{V_t}}}{2} \quad (5)$$

$$S_2 = \frac{-\left(K_r(1+\phi\gamma) + \frac{\lambda_f}{V_t}\right)}{2} - \frac{\sqrt{\left(K_r(1+\phi\gamma) + \frac{\lambda_f}{V_t}\right)^2 - 4\frac{\lambda_f K_r}{V_t}}}{2} \quad (6)$$

and

$$\phi = V_s/V_t \quad (7)$$

With C_0 and V_t known, and using the time series of solution measurements, the parameters λ_f/V_t, K_r, and $\phi\gamma$ were estimated using Equations 4 through 7 and a nonlinear least-squares optimization procedure [17,18]. Since V_t is known, λ_f was obtained. That value is a rate constant, with units of chemical uptake per time, and was used to compare uptake rates.

The second method of describing the uptake rate constant (λ_f) is based on the appearance of ^{14}C in various plant organs. To measure ^{14}C in plant tissues, the plants were periodically removed from the exposure chambers. The stem was cut at the crown and each part (roots and tops) was weighed and analyzed fresh for radioactivity by oxidation and LSC as previously described.

These data were used to calculate the approximate uptake rate, λ_f, using the total amount of chemical in roots or in plant tops over a short period in the following equation:

$$\lambda_f \cong \frac{M_r}{\int_0^t C_t(\tau)d\tau} \quad (8)$$

where M_r is the total amount of chemical in roots or tops at the time of sampling, and $C_t(\tau)dt$ is the chemical concentration in solution integrated over the time of exposure.

Chemical metabolism

Thin-layer chromatographs were made with extracts of plant tissues collected on the final day of experiments for three of the chemicals (bromacil, DNB and DCBN). This was done to determine if the ^{14}C in the plant was in the form of the parent chemical. Treatment chemicals were spotted on each TLC plate as a reference for locating the parent compound. Plants treated with bromacil were extracted in 30 ml ethanol for 2 min in a Virtis homogenizer. The slurry was centrifuged at 34,000 *g* for 15 min, and the solution decanted and dried in a rotoevaporator. The dried extract was redissolved in dichloromethane and spotted (30 μl) on Whatman LK6DF plates. These were eluted with either isoctane:dichloromethane (4:1, v/v) for 1 h or ether:hexane (1:1, v/v) for 4 h; after drying, they were used to expose Kodak NS2T x-ray film for 48 h. The spots were scraped from the chromatograph into paper thimbles that were oxidized; the resultant $^{14}CO_2$ was analyzed by LSC.

Plant tissues exposed to DNB and DCBN were extracted in 20 ml ethanol, similar to the method for bromacil. The slurry was centrifuged at 34,000 *g* for 20 min, after which the supernatant was decanted and the pellet resuspended two times in 10 ml ethanol, centrifuged and decanted. The centrifuged extracts were combined and made up to

50 ml with ethanol. The ethanol extracts were applied directly to chromatography plates in quantities ranging from 20 to 100 μl in 10-μl increments. The plates were eluted for 1 h with benzene:ethanol (5:1, v/v) and then dried. The dried chromatograph was used to expose Kodak XAR-5 x-ray film for 30 d. This was much longer than for bromacil because there was less activity in the unconcentrated extracts. Following exposure, the spots were recovered and analyzed by the same procedure used in the bromacil experiment.

RESULTS AND DISCUSSION

Uptake rate constants

Concentration patterns in the whole-plant experiment treatment solutions are shown in Figure 1. Since chemical loss from solution was due primarily to root uptake, the initial slope, if expressed on a common basis (percentage of initial concentration), can be used as an indication of relative uptake rates. From Figure 1, it is obvious that the chemicals were initially depleted from solution differently. These data were used in equations 4 through 7 of the model described in the methods section to estimate the rate constants for chemical uptake into plants. The calculated rate constants are shown in Table 3 ("Solution"), wherein replicate determinations are in adjacent rows. Differences between chemicals are clearly evident, with DNB and NB having larger uptake rate constants than the two herbicides DCNB and bromacil.

Initial plant uptake rate constants were also determined using Equation 8, which considers the amount of chemical measured in plant tissues. Since both methods have the same units and both indicate total chemical uptake in whole plants, they are comparable and should yield similar values.

Correlation between these values ("Plant" and "Solution") yielded a coefficient of 0.97, indicating that both methods of determining the uptake rate constant were similar. The value for "Tops" plus "Root" (Table 3) equals the value for "Plant" (they do not always balance because of rounding). Within the period of those calculations, root uptake ("Root") predominated in the movement of chemical in plants, with the rate constants for chemical movement into stems plus leaves ("Tops") being small in comparison. The values for the "Tops" also exhibit a larger variation between replicates. This is an artifact of the calculations,

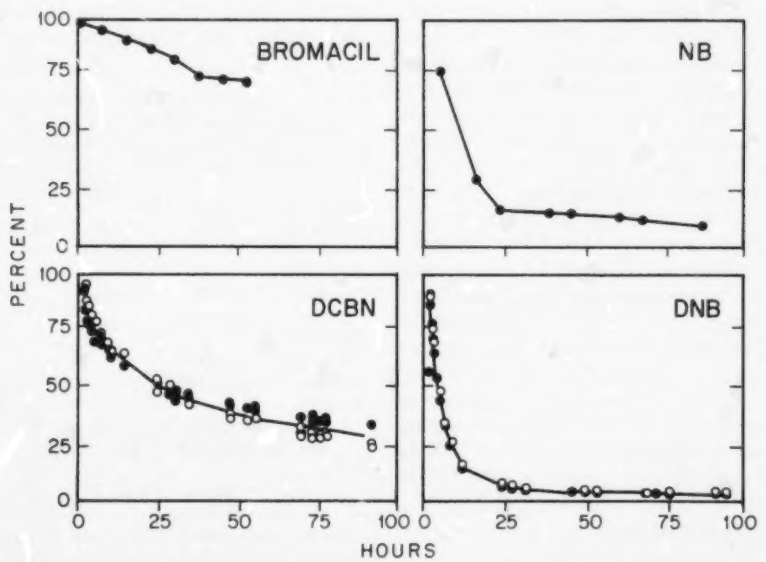


Fig. 1. Change in chemical concentration (% of initial ^{14}C value) in nutrient solution over time after an acute treatment with the following chemicals: bromacil,, nitrobenzene (NB), dichlorobenzonitrile (DCBN) and dinitrobenzene (DNB). Replicates are shown as ○ and ●. Bromacil and nitrobenzene were not replicated.

Table 3. Uptake rate constants of four test chemicals measured by the following methods: depletion from hydroponic solution in whole plant experiments (solution); appearance of ^{14}C in whole plants (Plant) and specified tissues (Root, Tops); and appearance of ^{14}C in isolated roots (IRUT)

	Uptake rate constants					
Chemical	Solution (ml min^{-1})	Plant (ml min^{-1})	Root (ml min^{-1})	Tops (ml min^{-1})	IRUT[a] (min^{-1})	SE
DCBN	2.5	2.4	2.4	0.0	0.3	0.13
Bromacil	1.8	3.9	2.7	1.3	0.7	0.04
	1.2	3.4	3.2	0.2		
DNB	18.0	22.2	21.8	0.4	0.9	0.05
	17.0	17.5	17.2	0.3		
NB	7.2	6.2	5.8	0.1	3.4	0.07

See Table 1 for abbreviations.
[a]Values represent the mean of six or more measurements.
[b]Not measured.

since the distribution of test chemical in the stems and leaves follows a regular pattern (as is discussed later).

The final column "IRUT" in Table 3 shows uptake rate constants determined with isolated barley roots in a considerably different procedure and yielding constants with different units. However, since the IRUT method measured initial root uptake and adsorption, we were curious to determine if the relationship between chemicals measured by IRUT and by whole-plant "Solution" and "Plant" were similar. The relative patterns (Table 3) generally hold for all of the chemicals but DNB. The reasons for this difference are not readily apparent but probably are an indication of species difference (barley vs. soybean) and the manner of testing. The whole-plant functions of transpiration, photosynthesis and within-plant translocation of chemical were absent in the IRUT. Without translocation between root and shoot via a transpirational stream, IRUT measures only cellular absorption, surface adsorption and symplastic solute movement. In intact plants, the apparent free space of the root is exposed to chemical not only by diffusion, as in the isolated roots, but also by mass flow of solution being moved by transpiration. Partitioning of chemical between liquid and solid phases in the xylem and movement to aerial organs may also play an important role in determining the initial uptake rate and thus contribute to the observed difference.

Chemical metabolism

Inherent in the presentation of data based on radioactivity analysis is the assumption that all measured ^{14}C represents the parent compound. This is clearly not the whole picture, since the test chemicals were in a rather reactive environment and subject to possible metabolism both in the nutrient solution and within the plants. To learn something about chemical degradation and persistence in the whole-plant tests, tissues of the last-sampled plants were extracted with solvents and the radioactivity was analyzed on thin layer chromatographs. Table 4 shows the percentage of activity recovered in each spot and the associated reference factors (R_f). In most cases the parent chemical was the predominant compound, with

Table 4. Reference front (R_f) values from thin-layer chromatographs and the percentage (%) of ^{14}C activity associated with each spot

		Unidentified metabolites		
Chemical and tissue	Parent R_f|%	R_f|%	R_f|%	R_f|%
Bromacil[a]				
Leaves	0.05|95	1.00|5		
Roots	0.05|100			
Bromacil[b]				
Leaves	0.40|95			
Roots	0.40|100			
DCBN[c]				
Leaves	0.90|53	0.70|24	0|23	
Roots	0.90|100			
DNB[c]				
Roots	0.94|58	0.25|10	0.07|7	0|24

The R_f values were obtained from chromatographing extracts from roots and leaves of soybean plants exposed in the exposure chambers. See Table 1 for abbreviations.
[a]Eluent = 4:1 (v/v) isooctane:dichloromethane.
[b]Eluent = 1:1 (v/v) ether:hexane.
[c]Eluent = 5:1 (v/v) benzene:ethanol.

different amounts of by-products showing as secondary spots.

Bromacil in leaves was recovered primarily as parent compound, with only one minor metabolite showing up in a TLC eluted with 4:1 isooctane: dichloromethane. That compound showed up as a smear from the origin to the wetting front, while the bromacil remained at the origin and accounted for 95% of the activity in the plant. With the ether:hexane elution a small spot appeared near the origin that was presumably the same material described above, while the bromacil moved up the column. No metabolites were detected in the root extracts.

DCBN was recovered as all parent compound in the roots, but two additional compounds appeared in the leaves. These metabolites accounted for about half the radioactivity in the leaves.

In the leaves of plants treated with DNB, the amount of ^{14}C was so small it could not be detected on the TLC, so metabolites in leaf tissue were not observed. However, the oxidation and liquid scintillation analysis of leaf tissue revealed that ^{14}C was in the leaves. The pattern from the root extracts indicated that about half the parent chemical was degraded to other compounds within the roots. Since there was no single band observed for the leaf samples, it was assumed that the radioactivity observed in the scintillation counting was due to metabolites similar to those found in the roots. This would account for the ^{14}C being spread out on the plate and thus undetectable.

Distribution

To determine the chemical movement within the plants, distribution patterns were measured in plants removed from the treatment solution over several days. Figure 2 presents the distribution on the basis of percentage of chemical within each plant, in the roots and tops. Bromacil was originally distributed in the roots and over time moved into the stems and leaves. After the first day the plants appeared to be in equilibrium with regard to the movement of bromacil.

A similar pattern occurred for DCBN in which the chemical fraction in the roots decreased while that in the tops increased. This indicated movement of the chemical from the roots through the stems to deposition in the leaves.

The distribution pattern of NB remained constant throughout the experiment, with about 95% being associated with the roots. DNB remained mostly in roots but ^{14}C was slowly translocated to the shoots, as indicated by the converging traces in Figure 2. Evidence from the TLC examination of the ^{14}C in the final leaves of the study (Table 4) indicates that the translocated chemicals were probably degradation products rather than DNB.

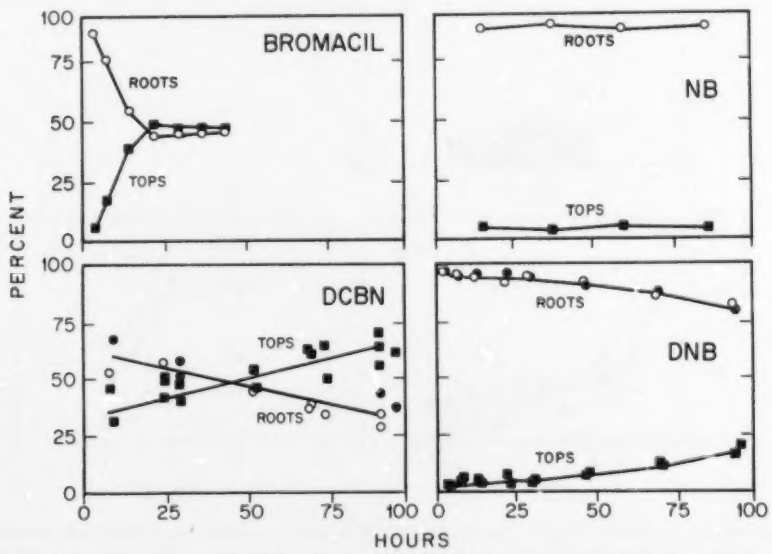


Fig. 2. Chemical distribution patterns with whole soybean plants shown as the percentage of all the chemical in the plants over time. Roots are indicated by open circles and tops by filled squares.

It is interesting to note that although the initial uptake rate constants for bromacil and DCBN (Table 3) were lower than for NB and DNB, the two herbicides moved into the stems and leaves more readily over the extended period of time. This accounts for their utility as herbicides that inhibit photosynthesis in leaves.

Rates of chemical uptake, chemical distribution patterns and sites of chemical deposition within plants are determined by a complex system of partitioning, permeability and metabolism for each chemical. An example of the distribution patterns of ^{14}C is shown for stems, petioles and leaves of plants treated with DCBN in Figure 3. The stem segments are between the nodes, starting at the crown and moving upward. The same notation was used for petioles and leaves, which were sampled and analyzed from nodes 4, 7 and 10. The lower lines represent the first sampling and the top lines, the last. The relative pattern of ^{14}C in the stems remained the same over time, with the lower stem segments always higher in concentration than the top segments. The petioles had a somewhat similar pattern, but contained higher concentrations than did their associated leaves and stem segments. This indicated that some barrier to movement of DCBN and/or its metabolites exists in the leaf symplasm, resulting in an accumulation of ^{14}C in the petiole.

Figure 4 presents an autoradiogram of ^{14}C activity in the seventh full leaf of a plant 5 d after treatment with DCBN. The radioactivity was restricted mainly to conductive tissue, but some appeared within the interveinal areas. Some leaves were surgically separated into veinal tissue (including the midvein and dominant lateral veins of each leaflet) and interveinal lamella, and both segments and the petiole were analyzed for ^{14}C activity. The results for a leaf adjacent to the one shown in Figure 4 were as follows: interveinal, 95; veinal, 243; petiole, 536 (×1,000 dpm/g dry wt.). These data and picture are consistent with the information presented in Figure 3, which suggests that DCBN is primarily restricted to vascular tissue and tends to accumulate at the distal end of that system (petioles and leaf veins).

CONCLUSIONS

These studies have shown the whole plant uptake, distribution and metabolism of selected xenobiotic chemicals in soybean plants grown in

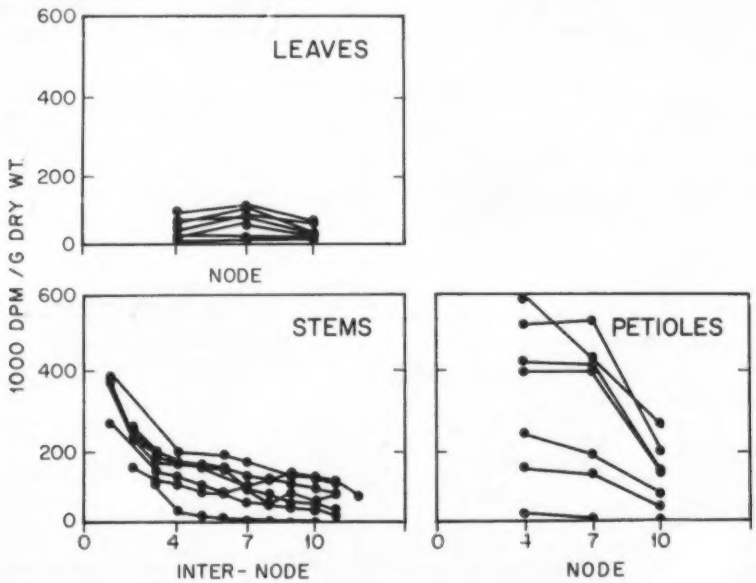


Fig. 3. Distribution patterns of dichlorobenzonitrile (DCBN) in soybean plants. The petioles and leaves were from the fourth, seventh and tenth nodes and the stem segments were internodal pieces. The lower lines represent plants harvested first and the top lines, those harvested last. Harvest times were at 7, 25, 31, 55, 73, 79 and 97 h.

Fig. 4. Autoradiograph of the three leaflets of the leaf taken from the fourth node of a soybean plant exposed to ^{14}C-labeled dichlorobenzonitrile (DCBN) for 5 d.

carefully controlled environments. The initial uptake rate constants determined by measuring either chemical loss from solution or chemical concentration in plants yielded similar results in the order: DCBN = bromacil < NB < DNB. Although the initial uptake rates were higher for DNB and NB, they were primarily restricted to roots. On the other hand, the two herbicides, DCBN and bromacil, had lower original uptake constants, but, in contrast, they continued moving within the plants.

The whole-plant uptake rates were compared with a tissue test based on isolated barley roots. The patterns of uptake rate constants were similar except for DNB, the reason for that deviation not being apparent.

Studies of metabolism of ^{14}C-labeled chemicals in plants indicated that DNB was metabolized in the roots, since about half of the ^{14}C was found in spots (by TLC) other than the parent compound in both root and leaf samples. DCBN was apparently metabolized in the leaves, since about half of the ^{14}C was found in other spots (by TLC) in leaf samples but all the ^{14}C was parent chemical in root samples. Bromacil was primarily present as parent compound in both leaves and roots.

It is clear from these studies that a formal kinetic description is needed to describe the data in a quantitative manner so that comparisons can be made between chemicals and plant species. To accomplish this task, we have chosen to identify the processes involved and use a mathematical model to describe the effects of diffusion, partitioning, mass flow and reflection at the various boundaries within plants. The experimental setup described here and the information retrieved in these experiments will become the basis for establishing that model.

Acknowledgement – We acknowledge the contributions made by Dr. Tom Lindstrom for help in determining the rate constants, and by Joel McCrady, Robert Alt, Karen Murchie, Jim Dray and Kim Hayden for their assistance with data reduction, analysis and sample preparation. Ms. Nolt was supported in part by the Ecotoxicology Development Grant from the A. W. Mellon Foundation to Cornell University and by the Edna Bailey Sussman Fund Internship Program.

REFERENCES

1. **Verloop, A.** 1972. Fate of the herbicide dichlobenil in plants and soil in relation to its biological activity. *Residue Rev.* **43**:55–103.
2. **Gortz, J.H.** and **J.L.P. Van Oorschot.** 1984. Uptake and translocation of ^{14}C asulam and ^{14}C bromacil by roots of maize and bean plants. *Pestic. Biochem. Physiol.* **21**:45–52.
3. **Callahan, M.A.** and **W.M. Slimak.** 1979. Water-

related environmental fate of 129 priority pollutants. EPA 440/4-79-029a. U.S. Environmental Protection Agency, Washington, DC.

4. **Tanton, T.W.** and **S.H. Crowdy.** 1972. Water pathways in higher plants II. Water pathways in roots. *J. Exp. Bot.* **23**:600–618.
5. **Crowdy, S.H.** 1973. Patterns and processes of movement of chemicals in higher plants. *Proceedings*, Seventh British Insecticide and Fungicide Conference, Brighton, England, November 19–22, 1973, pp. 831–839.
6. **Christ, R.A.** 1978. Physiological and physicochemical requisites for the transport of xenobiotics in plants. In *Fourth International Congress of Pesticide Chemistry: Advances in Pesticide Science*, Part 3. Pergamon Press, New York, NY, pp. 420–429.
7. **Moody, K., C.A. Kust** and **K.P. Buchholtz.** 1970. Uptake of herbicides by soybean roots in culture solutions. *Weed Sci.* **18**:642–647.
8. **McFarlane, C.** and **C. Wickliff.** 1985. Excised barley root uptake of several ^{14}C labeled organic compounds. *Environ. Monit. Assess.* **5**:385–391.
9. **Walker, A.** and **R.M. Featherstone.** 1973. Absorption and translocation of atrazine and linuron by plants with implications concerning linuron selectivity. *J. Exp. Bot.* **24**:450–458.
10. **Hartley, G.S.** and **I.J. Graham-Bryce.** 1980. Uptake into roots and xylem. In *Physical Principles of Pesticide Behavior*, Vol. 2. Academic Press, New York, NY, pp. 596–611.
11. **Crowdy, S.H.** and **D. Rudd Jones.** 1956. The translocation of sulphanamides in higher plants. I. Uptake and translocation in broad beans. *J. Exp. Bot.* **7**:335–346.
12. **Briggs, G.E.** and **R.N. Robertson.** 1957. Apparent free space. In A.S. Craft, ed. *Annual Review of Plant Physiology*, Vol. 8. Annual Reviews, Inc., Palo Alto, CA, pp. 11–30.
13. **Shone, M.G.T.** and **A.V. Wood.** 1973. Pathways of uptake and translocation of some soil-applied pesticides. *Proceedings*, Seventh British Insecticide and Fungicide Conference, Brighton, England, November 19–22, 1973, pp. 151–159.
14. **Wickliff, C., J.C. McFarlane** and **H. Ratsch.** 1984. Uptake of bromacil by isolated barley roots. *Environ. Monit. Assess.* **4**:43–51.
15. **McFarlane, C.** and **T. Pfleeger.** 1986. Plant exposure laboratory and chambers. EPA 600/3-86/007a,b. U.S. Environmental Protection Agency, Corvallis, OR.
16. **Berry, W.L.** 1978. Nutrition, containers, and media. In R.E. Langhans, ed., *A Growth Chamber Manual*. Cornell University Press, Ithaca, NY, pp. 117–135.
17. **Lindstrom, F.T.** 1979. An objective function surface exploration algorithm. Technical report No. 69. Department of Statistics, Oregon State Univeristy, Corvallis, OR.
18. **Lindstrom, F.T.** 1980. FUNMIN: An outline-interactive, mixed simplex-Marquardt least squares of likelihood minimization algorithm. Technical Report No. 73. Department of Statistics, Oregon State University, Corvallis, OR.

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.